Cell

In Vitro Analysis of Huntingtin-Mediated Transcriptional Repression Reveals Multiple Transcription Factor Targets

Weiguo Zhai,[1] Hyunkyung Jeong,[2] Libin Cui,[2] Dimitri Krainc,[2,*] and Robert Tjian[1,*]
[1] Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, 401 Barker Hall, Berkeley, CA 94720, USA
[2] Department of Neurology, Massachusetts General Hospital, Harvard Medical School, MassGeneral Institute for Neurodegenerative Disease, 114 16th Street, Charlestown, MA 02129, USA
*Contact: krainc@helix.mgh.harvard.edu (D.K.); jmlim@uclink4.berkeley.edu (R.T.)
DOI 10.1016/j.cell.2005.10.030

SUMMARY

Transcriptional dysregulation has emerged as a potentially important pathogenic mechanism in Huntington's disease, a neurodegenerative disorder associated with polyglutamine expansion in the huntingtin (htt) protein. Here, we report the development of a biochemically defined in vitro transcription assay that is responsive to mutant htt. We demonstrate that both gene-specific activator protein Sp1 and selective components of the core transcription apparatus, including TFIID and TFIIF, are direct targets inhibited by mutant htt in a polyglutamine-dependent manner. The RAP30 subunit of TFIIF specifically interacts with mutant htt both in vitro and in vivo to interfere with formation of the RAP30-RAP74 native complex. Importantly, overexpression of RAP30 in cultured primary striatal cells protects neurons from mutant htt-induced cellular toxicity and alleviates the transcriptional inhibition of the dopamine D2 receptor gene by mutant htt. Our results suggest a mutant htt-directed repression mechanism involving multiple specific components of the basal transcription apparatus.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by psychiatric, cognitive, and motor abnormalities. Pathogenesis is associated with an expansion in the number of glutamine residues located in the amino (N) terminus of huntingtin (htt), a very large protein found mainly in the cytoplasm (DiFiglia et al., 1995; The Huntington's Disease Collaborative Research Group, 1993). Polyglutamine (polyQ) expansion in the mutant protein leads to its aberrant proteolytic cleavage, resulting in the release of N-terminal fragments that readily enter the nucleus (DiFiglia et al., 1997). These glutamine-rich mutant htt fragments are thought to contribute to the formation of nuclear inclusions and neurotoxicity and possibly activate an apoptotic cascade (Saudou et al., 1998). These processes have been experimentally modeled in cell culture and in transgenic animals expressing mutant htt (Sipione and Cattaneo, 2001). Importantly, the length of the polyQ tract in htt has also been correlated with the age of onset and the severity of symptoms in HD patients (Rubinsztein et al., 1993).

Although a number of different cellular events have been shown to associate with HD occurrence, the actual pathogenic mechanisms remain unclear. Recent evidence suggests that mutant htt may disrupt normal transcriptional programs in susceptible neurons during initial stages of HD pathogenesis, implicating transcriptional dysregulation as a potential pathogenic mechanism (Cha, 2000; Sugars and Rubinsztein, 2003). It has been well established, first in the case of Sp1 (Courey and Tjian, 1988) and subsequently with other transcription factors, that activation domains are often composed of glutamine-rich protein interfaces (Gerber et al., 1994). Thus, transcription factor interactions with other cellular factors may be disrupted by mutant htt bearing polyQ expansions. Indeed, mutant htt has been shown to interact directly with a number of nuclear transcription factors (Okazawa, 2003). Recent DNA microarray studies detected changes in gene expression profiles in HD transgenic mice at early stages, suggesting that transcription of select genes had already been altered even when mice showed only minimal abnormalities (Luthi-Carter et al., 2002a; Luthi-Carter et al., 2002b). Analysis of the affected regulatory sequences revealed that select Sp1-dependent transcription pathways were disrupted. This hypothesis is strongly supported by recent in vivo observations that mutant htt may target Sp1 and its coactivator TAF4 (formerly $TAF_{II}130$; Chen et al., 1994; Tanese et al., 1991) through direct protein-protein interactions to disrupt transcription (Dunah et al., 2002). Remarkably, in primary striatal neurons, coexpression of Sp1

and TAF4 resulted in a significant rescue of mutant htt-induced inhibition of dopamine D2 receptor gene (*D2*) promoter activity (Dunah et al., 2002).

Human TAF4 is one of at least 12 TATA binding protein (TBP)-associated factors (TAF_{II}s) in TFIID (Albright and Tjian, 2000). The transcription initiation factor TFIID is recruited to the core promoter through its interaction with specific activators such as Sp1 and binds to the TATA element at core promoters. A series of transcription factor interactions, involving TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, CRSP (also called mediator), and RNA polymerase II (Pol II) subsequently leads to the formation of the preinitiation complex (PIC) and transcriptional activation (Ryu et al., 1999). Human TFIIF consists of two subunits, RAP30 and RAP74, that bind RNA Pol II directly and help recruit the enzyme to a preformed TFIID/TFIIB complex (Conaway et al., 2000).

Although cell-based assays have provided important clues to the potential relationship between htt, Sp1, and TAF4, it remains unclear whether mutant htt interferes directly with Sp1, TFIID, or potentially other components of the PIC to repress transcription. To determine the molecular mechanisms employed by mutant htt to repress transcription, the biochemical properties and specificity of this glutamine expansion protein may best be revealed by using purified proteins and an integrated in vitro transcription reaction responsive to putative htt targets, such as Sp1 and TFIID.

Here, we report the development of a well-defined chromatin based in vitro transcription assay consisting of highly purified factors to study the molecular mechanisms of mutant htt-mediated transcriptional repression. Various N-terminal fragments of htt carrying either a normal or an expanded polyQ tract were tested for their ability to repress transcription in Sp1-dependent versus Sp1-independent in vitro reconstituted reactions. Using this highly purified in vitro assay, we identified several specific factors targeted by mutant htt to repress transcription. In vitro and in vivo protein binding assays revealed a direct interaction between htt and distinct components of the PIC. Importantly, these interactions were sensitive to the length of the polyQ expansion. The role of these factors in transcriptional repression and HD pathogenesis was further verified in cultured primary striatal neurons. These studies reveal that specific components of the core transcriptional apparatus are directly targeted by soluble forms of mutant htt that operate as a selective repressor or corepressor to disrupt normal gene expression in HD cells.

RESULTS

Mutant htt Specifically Represses Sp1-Dependent Transcription In Vitro

We adopted a chromatin-based in vitro transcription system (Lemon et al., 2001; Naar et al., 1999) to dissect potential mechanisms by which mutant htt represses Sp1-dependent transcription. The Sp1 transcription template was first assembled into chromatin using *Drosophila* S190 and purified core histones. As expected, high levels of transcription from the assembled chromatin template were obtained with purified recombinant Sp1 and a well-defined set of basal transcription factors including purified recombinant TFIIA, TFIIB, TFIIE, TFIIF and affinity-purified TFIID, TFIIH, RNA Pol II, and CRSP (Figure 1A, lane 1). To assess the effect of mutant htt on Sp1-dependent transcription, we expressed the N-terminal portion (1–171 aa) of either wild-type (wt; Htt-23QP) or mutant (Htt-120QP) htt in *E. coli* and purified these proteins using Ni-NTA affinity chromatography (Figure 1D). As shown in Figure 1A, adding wt htt (up to 0.3 pmol) did not significantly alter the levels of transcription (lanes 2–5). In contrast, adding 0.1–0.3 pmol of mutant htt resulted in a dramatic decrease in transcription (Figure 1A, lanes 8 and 9). Thus, purified recombinant mutant htt fragment is capable of inhibiting Sp1-dependent transcription in a well-defined in vitro reaction, and this repression appears to depend on the presence of an expanded polyQ in htt.

Next, we examined whether this repression by mutant htt is specific for Sp1-dependent transcription by assaying its effect on an unrelated "acidic" activator, Gal4-VP16. Transcription from the Gal4 chromatin template is highly dependent on the presence of Gal4-VP16 (Figure 1B, compare lanes 1 and 2). In contrast to the results obtained from Sp1-dependent transcription assays, adding mutant htt to a level that efficiently repressed Sp1 activation had no significant effect on Gal4-VP16-mediated transcription (Figure 1B, lanes 3–5). However, if much higher amounts of mutant htt were used, some repression even with VP16 can be observed (data not shown). These findings suggest that the htt directed repression observed in vitro is rather specific for certain activators such as Sp1 and not likely due to nonspecific aggregation or global inhibition of activators.

It has been reported that htt protein can bind to the acetyltransferase (HAT) domain of CREB binding protein (CBP) and p300/CBP-associated factor (P/CAF) and inhibit their HAT activity (Steffan et al., 2001). Therefore, it is possible that the htt-directed transcriptional repression we observed was a consequence of mutant htt-mediated interference with chromatin modifying or remodeling activities present in our assay system. To address this issue, we performed in vitro transcription assays using naked DNA templates and completely purified transcription components. As shown in Figure 1C, adding purified mutant htt efficiently reduced the levels of Sp1-dependent transcription even on naked DNA templates while addition of wt htt at these levels had no effect. Because there is no chromatin modifying and remodeling required for transcription from naked DNA templates, the observed repression in vitro is unlikely to result from its interference with chromatin modifying and/or remodeling activities. This is also consistent with our observation that addition of the histone deacetylase (HDAC) inhibitor TSA had little or no effect on mutant htt-mediated transcriptional repression in our assay (data not shown).

Rescue of Transcriptional Repression Mediated by Mutant htt In Vitro

To better understand the mechanisms of transcriptional repression by mutant htt, we decided to systematically test

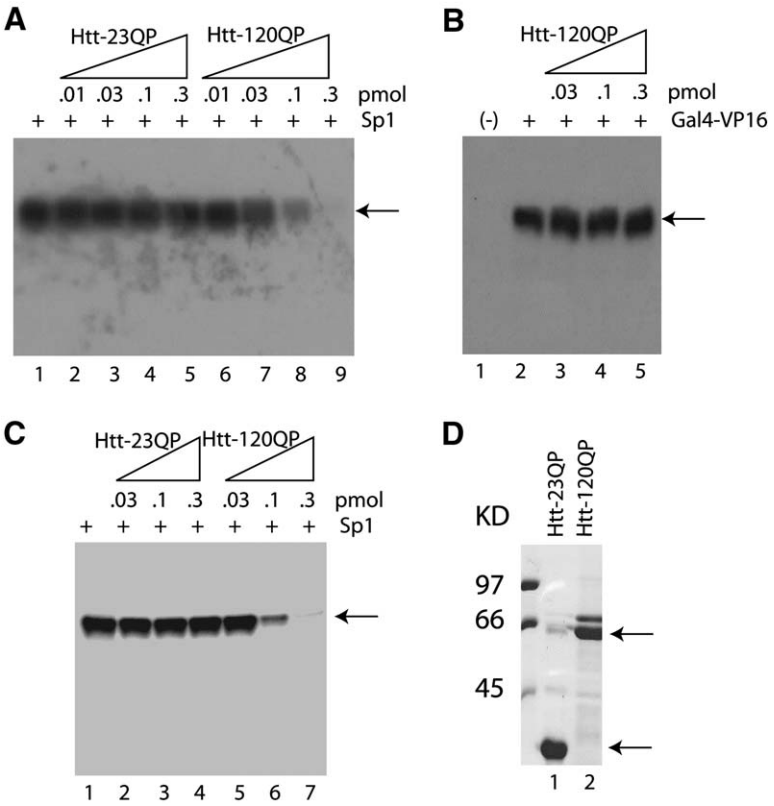

Figure 1. Development of an In Vitro Transcription System Responsive to Mutant htt

(A) Mutant htt inhibits Sp1-dependent transcription in vitro. In vitro transcription was directed from the Sp1 chromatin template using purified Sp1 and basal transcription factors either in the absence or presence of normal htt (Htt-23QP) or mutant htt (Htt-120QP).

(B) Mutant htt does not inhibit Gal4-VP16-dependent transcription from the Gal4 chromatin template in vitro.

(C) Mutant htt inhibits Sp1-dependent transcription in vitro from naked DNA template.

(D) His_6-tagged htt fragments (1–171 aa) containing 23 or 120 polyQ (Htt-23QP and Htt-120QP) were visualized by Coomassie staining.

which components present in our defined in vitro transcription system could rescue the inhibition caused by mutant htt. It has been reported that mutant htt can disrupt the interaction between Sp1 and its coactivator TAF4 and that the repression of the *D2* promoter activity by mutant htt can be reversed by coexpression of Sp1 and TAF4 in cultured striatal cells (Dunah et al., 2002). Based on these in vivo results, we anticipated that addition of Sp1 and/or TAF4 (in the form of TFIID complex) should rescue the transcriptional repression induced by mutant htt. For these experiments, we chose to use an amount of mutant htt (Htt-120QP, 0.15 pmol) that would repress transcription by about 80%–90% (Figure 2A, compare lanes 1 and 2). We also used the native holo-TFIID instead of the isolated TAF4 subunit because the purified recombinant protein is poorly behaved and has a tendency to aggregate or becomes proteolyzed into smaller fragments. To rescue this level of repression, an additional 2- to 4-fold of each individual basal factor was added to transcription reactions containing mutant htt. As expected, addition of purified Sp1 (Figure 2A, lanes 19 and 20) or the TAF4-containing TFIID complex (Figure 2A, lanes 7 and 8) was able to efficiently rescue the repression caused by mutant htt. These findings indicate that our in vitro transcription system largely recapitulates the transcriptional repression observed for mutant htt in vivo. In addition, they provide evidence that most likely both Sp1 and TFIID are directly targeted by mutant htt for repression.

To screen for potential novel targets of mutant htt, we also attempted to rescue the in vitro repression by adding other components of the core transcription machinery. Surprisingly, we found that addition of excess TFIIF can reverse the repression (Figure 2A, lanes 11 and 12), suggesting that TFIIF may also be targeted by mutant htt for repression. Addition of the other basal factors and cofactors had little or no effect on the repression by mutant htt (Figure 2A). As a control, we also added the same amounts of excess individual factors to normal transcription reactions containing no mutant htt. As shown in Figure 2B, addition of excess factors to our in vitro transcription system had little effect on the overall transcription level, suggesting that under our standard transcription condition, the level of basal factors used are already saturating for transcription initiation.

The Length of polyQ Expansion Correlates with Repressor Potency

A key observation made in HD patients is that longer polyQ expansions in htt are associated with more severe symptoms and earlier age of onset (Rubinsztein et al., 1993). Therefore, it is important to determine whether mutant htt with varying polyQ length would also differ in their ability to repress transcription in vitro. To test this potential correlation, we expressed and purified His_6-tagged htt exon1 carrying various numbers of polyQ (Htt-25QP, Htt-46QP, and Htt-97QP; Figure 3A). Purified proteins that remained soluble after dialysis were resolved by SDS-PAGE, and their relative concentrations were estimated by immunoblotting using antibodies against the His_6 tag. When these purified htt polypeptides were tested in transcription assays, their ability to

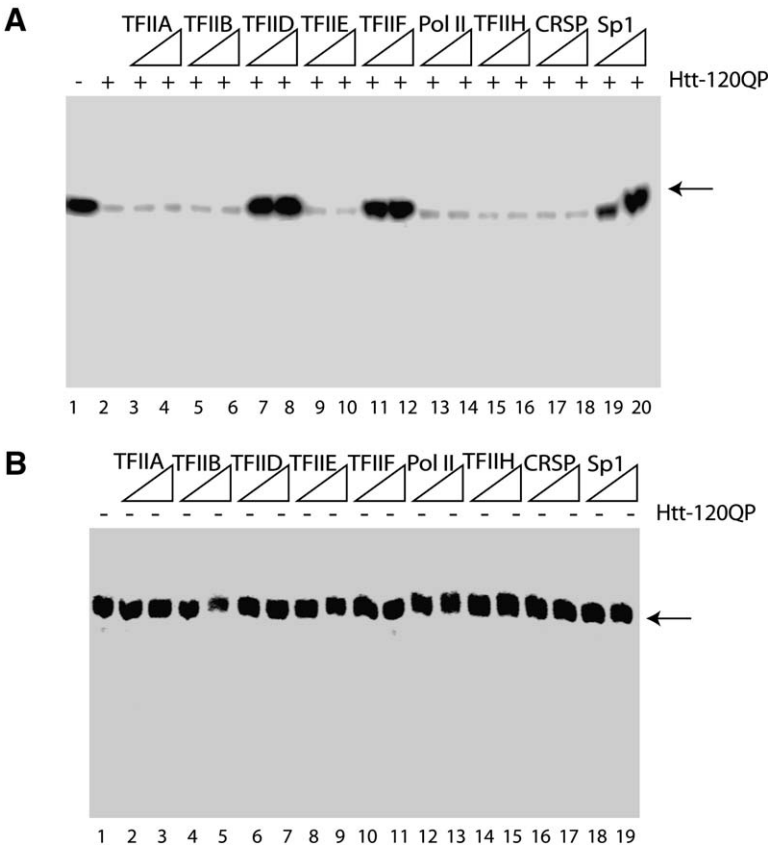

Figure 2. Rescue of Mutant htt-Mediated Transcriptional Repression

(A) Select basal transcription factors can rescue mutant htt-mediated transcriptional repression in vitro. An extra 2 (odd-numbered lanes)- or 4-fold (even-numbered lanes) of each factor (as labeled) was added to transcription reactions containing Htt-120QP.

(B) Effect of additional basal transcription factors on overall transcription in the absence of mutant htt.

repress transcription differed substantially. The addition of normal htt fragment (Htt-25QP; Figure 3B, lanes 2–5) had no measurable effect on Sp1-dependent transcription, while 100 pmol of mutant htt (Htt-46QP) inhibited transcription (Figure 3B, lane 9). When the number of polyQ in htt was increased further, its ability to repress transcription increased dramatically. The addition of 1.0 pmol of Htt-97QP resulted in a 90% decrease in the levels of transcription (Figure 3B, lane 12). Even lower amounts of Htt-120QP were sufficient to repress transcription efficiently (Figure 3B, lanes 13–15).

Next, we determined whether transcriptional repression by mutant htt carrying different numbers of polyQ targeted the same components of the transcription apparatus. As shown in Figure 3C, transcriptional repression by Htt-97QP (1.0 pmol) can be rescued by adding more TFIIF, TFIID, and Sp1. Addition of other basal transcription factors was not able to alleviate the repressive effect of this mutant htt (Figure 3C). Likewise, repression by Htt-46QP (100 pmol) can also be rescued by the addition of more TFIIF, TFIID, and Sp1 but not the other basal transcription factors (Figure 3D). Thus, the same set of factors (i.e., Sp1, TFIID, and TFIIF) was capable of reversing the transcriptional repression mediated by mutant htt carrying different numbers of polyQ. These results suggest that mutant htt with different numbers of polyQ likely utilizes the same molecular targets and mechanisms to repress transcription.

Interplay between Mutant htt and TFIIF

The data we obtained thus far strongly suggest that mutant htt can inhibit Sp1-dependent transcription by interfering with the functions of Sp1 and TFIID, as well as TFIIF. The ability of Sp1 and TFIID to rescue the transcriptional repression was expected since mutant htt can interact with Sp1 and TAF4 to disrupt this activator-coactivator pair in vivo (Dunah et al., 2002). Therefore, we sought to determine whether TFIIF could be targeted by mutant htt in a similar manner.

To investigate whether htt is able to interact with TFIIF (composed of RAP30 and RAP74) under normal physiological conditions, coimmunoprecipitation (coIP) studies were performed using extracts prepared from mouse striatum. As shown in Figure 4A, αRAP30 antibody was able to IP htt protein from knockin (KI) mice (Heterozygous, wt/140Q, Menalled et al., 2003) striatum extracts (lane 3) but not from normal (wt) mice striatum extracts (lane 7). In control reactions, IgG alone (Figure 4A, lanes 4 and 8) did not bring down any detectable htt protein. These data suggest that mutant htt can form a complex with RAP30 in HD striatum in a manner that is polyQ dependent and stable enough to coIP from crude cell extracts.

To further confirm this observation and also examine which subunit of TFIIF interacts with htt, we cotransfected COS7 cells with constructs encoding myc-tagged normal htt (Htt480-17, N-terminal 480 aa) or mutant htt (Htt480-68)

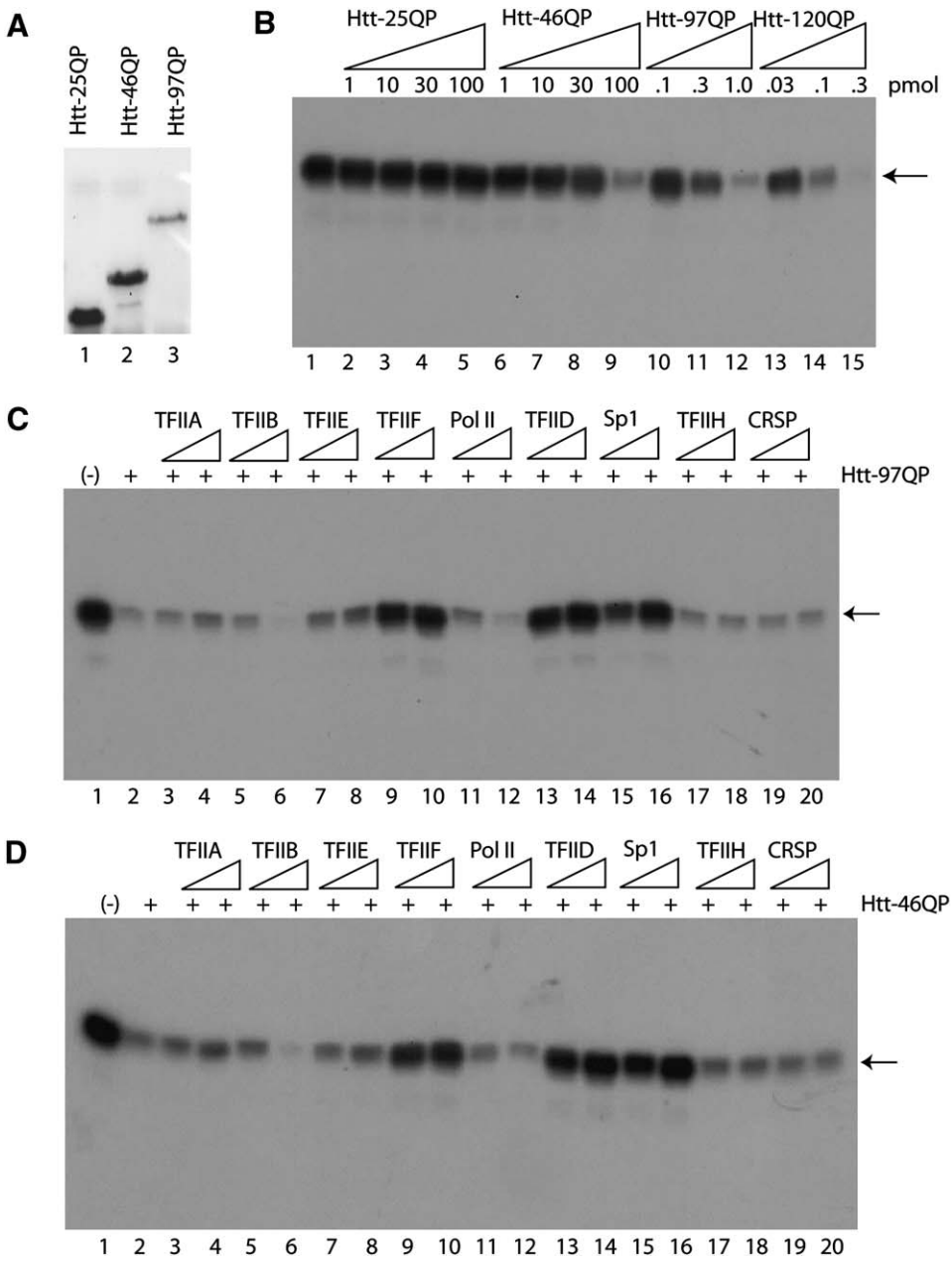

Figure 3. Correlation of the Length of polyQ Tract in htt with Its Ability to Repress Transcription

(A) His-tagged htt exon 1 containing 25, 46, or 97 polyQ (Htt-25QP, Htt-46QP, and Htt-97QP) were visualized by Coomassie staining.

(B) In vitro transcription reactions were carried out either in the absence or presence of purified Htt-25QP, Htt-46QP, Htt-97QP, or Htt-120QP.

(C) Rescue of transcriptional repression mediated by Htt-97QP.

(D) Rescue of transcriptional repression mediated by Htt-46QP.

together with Flag-tagged RAP30. The expression levels for wt and mutant htt in transfected cells were comparable (Figure 4B, input). CoIP with αFlag antibodies showed that RAP30 interacted strongly with mutant htt (Figure 4B, lane 4) while displaying only minimal affinity for wt htt (lane 3) over background (lanes 1 and 2). Reverse IP using the same lysates with αmyc antibodies to precipitate htt confirmed that the interaction between RAP30 and htt is polyQ dependent (Figure 4B, lanes 5 and 6). In contrast, RAP74 showed no detectable affinity for either wt or mutant htt in a similar experiment (Figure 5A, lanes 5 and 6). Surprisingly, when we cotransfected these cells with expression constructs for htt and RAP30 as well as RAP74, the htt-RAP30 interaction was severely reduced even though there was

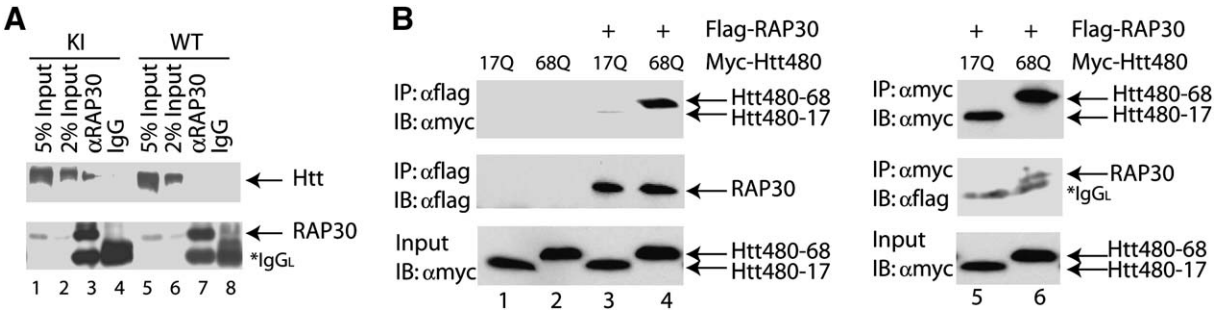

Figure 4. PolyQ-Dependent Interaction between htt and RAP30

(A) Increased interaction between htt and RAP30 in HD mouse striatum. Extracts prepared from either mutant htt knockin (KI, heterozygous, wt/140Q) mice striatum or normal (wt) mice striatum were immunoprecipitated with αRAP30 antibody and control IgG and subsequently immunoblotted with αhtt (top) or αRAP30 antibodies (bottom).

(B) COS7 cells were cotransfected with Flag-tagged RAP30 and myc-tagged htt constructs (1–480 aa, Htt480-17 or Htt480-68). The cell lysates were immunoprecipitated (IP) and subsequently immunoblotted (IB) with either αmyc or αFlag antibodies. Inputs of htt were detected using αmyc antibodies.

more RAP30 protein in cells cotransfected with RAP74 (Figure 5A, compare lanes 3 and 4 to 1 and 2). CoIP using cell lysates with a nontagged version of RAP74 yielded the same result (data not shown). Thus, it appears that RAP74 can efficiently compete with htt for binding to RAP30, suggesting that the interactions between RAP30-RAP74 and RAP30-htt are likely mutually exclusive.

Since RAP30 is thought to use its N-terminal region to bind RAP74 (Tan et al., 1995), we reasoned that RAP30 is likely to use the same or an overlapping domain for htt interaction. To directly test this idea and map the regions of RAP30 required for RAP74 and htt interactions, we assayed a series of Flag-tagged RAP30 deletion mutants in cotransfection and coIP experiments. As shown in Figure 5B (top panels), deletion mutants of RAP30 containing its first 90 or 60 amino acids interacted with RAP74 as efficiently as the full-length protein (lanes 1–3). However, further removal of N-terminal amino acids of RAP30 (1–40 aa) resulted in a severe loss of RAP74 interaction (Figure 5B, lane 4). As expected, mutants of RAP30 lacking various regions of the N terminus (40–249 aa and 60–249 aa) were unable to interact with RAP74 (Figure 5B, lanes 5 and 6), suggesting that the first 60 amino acids of RAP30 are minimally required for RAP74 interaction. The same set of RAP30 deletion mutants were also tested for their ability to interact with mutant htt (Htt480-68). As shown in Figure 5B (bottom panels), truncations of RAP30 containing its first 90 or 60 amino acids retained strong affinity for htt (lanes 7–9). Deletion mutants of RAP30 (1–40 aa and 60–249 aa, Figure 5B, lanes 10 and 11), which had lost their ability to interact with RAP74, also showed no detectable affinity for mutant htt. However, a RAP30 mutant lacking the first 40 amino acids of its N terminus but containing the remainder of RAP30 protein (40–249 aa) showed significant affinity for mutant htt despite its inability to interact with RAP74 (Figure 5B, lane 12). These domain-mapping studies that helped identify the interacting regions of RAP30 are summarized in Figure 5C. From these studies, we conclude that an N-terminal region of RAP30 (40–60 aa) is required and sufficient for mutant htt interaction. This same region is also required but not sufficient for RAP74 interaction. RAP74 appears to interact with more RAP30 N-terminal sequences that lie between amino acids 1 and 40. Thus, RAP30 appears to use overlapping domains for its interaction with RAP74 and mutant htt, providing us with a molecular rationale for the observation that RAP30 binding to htt and RAP74 are mutually exclusive.

To further confirm that the interaction between htt and RAP30 is direct, we performed GST pull-down experiments using GST-fusion htt exon 1 proteins (Figure 5D, lanes 10–12) and bacterial lysates expressing RAP30. As shown in Figure 5D, GST-fusion htt protein can selectively bind RAP30, whereas GST alone does not (lanes 1–4). As expected, mutant htt is somewhat more efficient in binding RAP30 than wt htt. In contrast, GST-htt did not pull down any RAP74 from bacterial lysates expressing RAP74 (Figure 5D, lanes 5–8). These results are consistent with our previous coIP data and confirm that the interaction between RAP30 and htt is direct.

Our coIP experiments showed that mutant htt might compete with RAP74 for binding to RAP30, likely affecting formation of the TFIIF complex. To test whether mutant htt can interfere with the TFIIF complex, we performed GST pull-down experiments with highly purified recombinant TFIIF complex (Figure 5D, lane 9) and GST-htt proteins. As shown in Figure 5D (bottom panels), GST mutant htt pulled down significantly more RAP30 than wt htt (Figure 5D, compare lanes 15 and 16). In contrast, very little RAP74 was pulled down (Figure 5D). Because a significant proportion of the RAP30 and RAP74 are in complex with each other in our TFIIF preparations, the presence of RAP30 in these pull downs likely reflects binding between htt and a pool of free RAP30 subunits dissociated from intact TFIIF. Thus, we envision that the way in which the RAP30-htt interaction could interfere with the formation of active TFIIF is by sequestering RAP30.

Having found that RAP30 interacts with htt primarily through its N-terminal RAP74 binding domain, we sought to determine the features of htt that are important for this binding. We first tested whether the proline-rich region and sequences further downstream could affect this interaction.

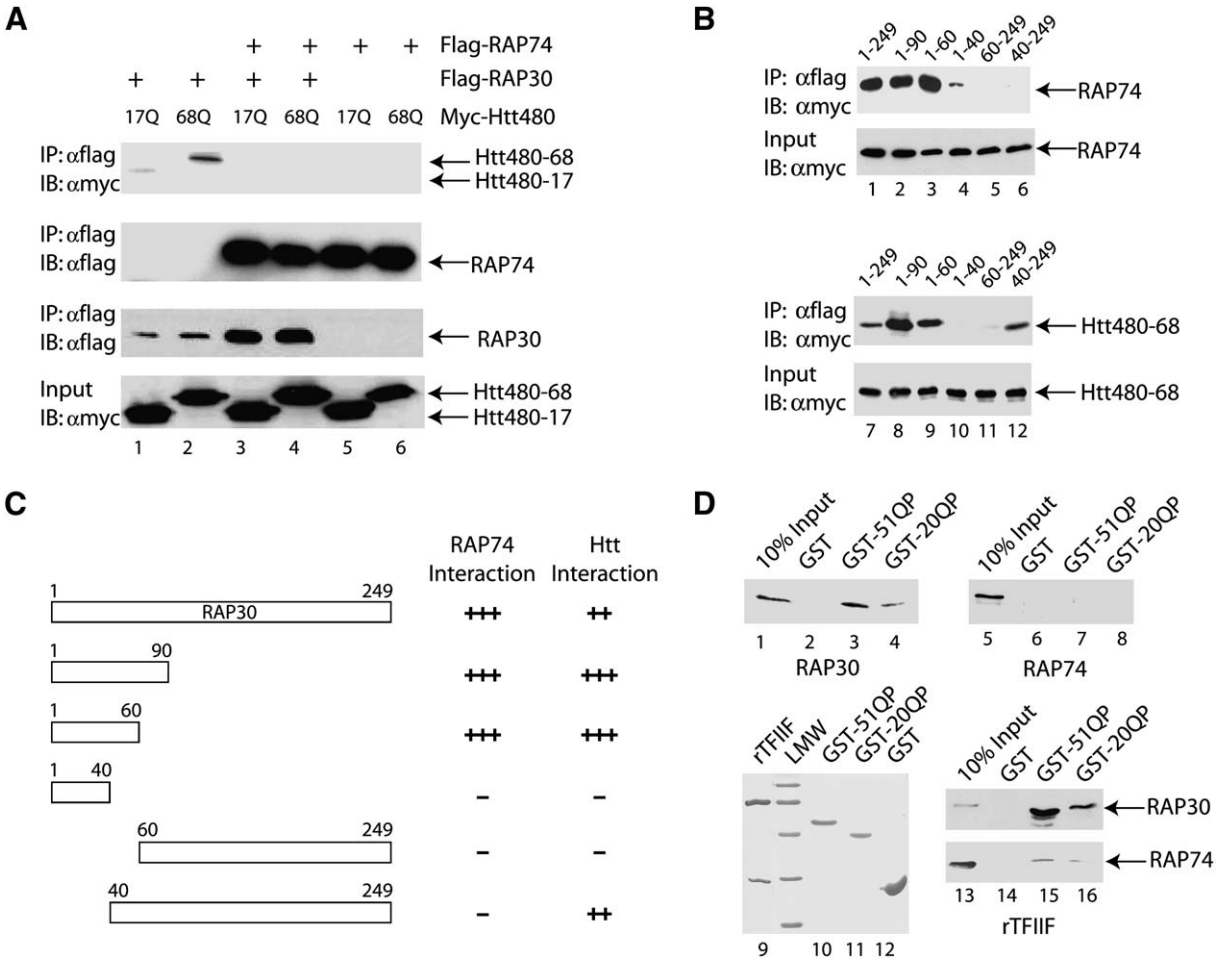

Figure 5. Mutually Exclusive Interactions between RAP30-RAP74 and RAP30-htt

(A) RAP74 competes with mutant htt for binding to RAP30. Lysates from cotransfected COS7 cells were immunoprecipitated and subsequently immunoblotted with αmyc and αFlag antibodies. Inputs of myc-htt were detected using αmyc antibodies.

(B) The N-terminal region of RAP30 is required for its interaction with both RAP74 and mutant htt. Flag-tagged RAP30 deletion mutants (including amino acids as labeled) were cotransfected into COS7 cells with either myc-RAP74 or myc-Htt480-68. The cell lysates were immunoprecipitated using αFlag resin followed by immunoblotting with αmyc antibodies.

(C) A schematic summary of the functional domain mapping results as described in (B).

(D) GST-htt interacts directly with RAP30 and pulls down RAP30 from intact TFIIF. GST-htt was able to pull down RAP30 (top left) but not RAP74 (top right). The purified recombinant TFIIF (rTFIIF) and GST-fusion proteins used in these pull downs were visualized by Coomassie staining (bottom left). Immunoblotting with αRAP30 and αRAP74 antibodies were used to detect their presence in pull downs (bottom right).

As shown in Figure 6, GST fusions that also contain the proline-rich domain (GST-20QP and GST-51QP), and downstream sequences (GST-171-20QP and GST-171-51QP) interact efficiently with RAP30. In contrast, their counterparts without the proline-rich domain (GST-20Q and GST-51Q) showed little or no affinity for RAP30. The presence of the residues immediately C-terminal to the proline-rich region did not significantly enhance the ability of htt to bind these three target factors. Thus, the proline-rich region in htt appears to be an important element for the in vitro interaction with RAP30. Interestingly, the proline-rich domain in htt also contributes to its interaction with TAF4 and Sp1 (Figure 6B). The finding that the same polyQ and proline-rich regions play critical roles in the interaction between htt and all three factors as well as CBP (Steffan et al., 2001) suggests that mutant htt may utilize similar or overlapping interfaces to interfere with transcription involving different transcription pathways. Quantitation of the data revealed that Htt interactions with RAP30 and Sp1 are significantly enhanced by polyQ expansion, whereas its interaction with TAF4 appears to be less affected by the number of glutamines.

Rescue of Mutant htt-Mediated D2 Promoter Inhibition by RAP30

To determine the potential relevance of RAP30 in mutant htt-mediated transcriptional repression in vivo, we investigated the transcriptional rescue of the *D2* promoter in striatal neurons. When a *D2* promoter reporter construct was

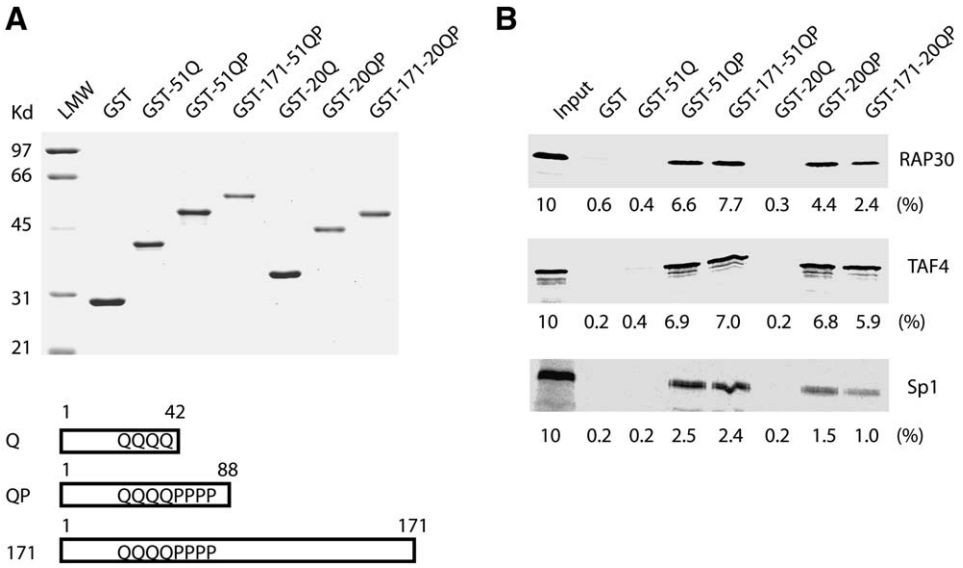

Figure 6. Both the polyQ and Proline-Rich Domain in htt Are Required for Its Interaction with RAP30, TAF4, and Sp1

(A) GST and various GST-fusion htt fragments used in pull downs were visualized by Coomassie staining.

(B) GST pull downs of [^{35}S]-methionine-labeled RAP30, TAF4, and Sp1 were analyzed by SDS-PAGE, developed, and quantitated (shown as percentage of input) with a PhosphorImager screen.

cotransfected together with htt into primary striatal neurons, wt htt (Htt480-17) had no observable repressive effect whereas mutant htt (Htt480-68) consistently resulted in a 2- to 3-fold inhibition (Figure 7A). Meanwhile, the NMDA receptor 1 (*NR1*) promoter, which is known not affected in HD, was not repressed under the same experimental conditions (data not shown). Importantly, when the RAP30 subunit alone or together with RAP74 was cotransfected into primary striatal neurons, the *D2* promoter inhibition was alleviated (Figure 7A). As a control, expression of RAP30 subunit alone or together with RAP74 in the absence of mutant htt had no significant effect on the *D2* promoter activity (Figure 7A). To exclude the possibility that the transcription rescue we observed at the *D2* promoter was caused by an altered expression level of htt protein as a result of added RAP30 and RAP74, we performed an immunoblotting assay to measure the protein levels of transfected htt and TFIIF subunits under the same assay conditions. As shown in Figure 7B, RAP30 and RAP74 expression did not change the protein levels of either wt or mutant htt (compare lanes 1–2 and 3–4). In addition, neither wt nor mutant htt coexpression had any significant effect on the expression level of RAP30/RAP74 in primary neurons (compare lanes 3 and 4 to 5). It should also be noted that no obvious cell toxicity was observed at the time of reporter activity analysis. Taken together, these cell-based assays corroborate our in vitro findings and suggest that TFIIF is a contributing factor involved in transcriptional inhibition mediated by mutant htt.

Rescue of Mutant htt-Induced Cellular Toxicity by RAP30

To correlate our transcription and protein interaction findings with possible HD pathogenic mechanisms in vivo, we investigated the potential relationship of TFIIF subunits and mutant htt in a cellular toxicity assay. Previous experiments demonstrated that mutant htt is toxic when overexpressed in cultured primary striatal neurons (Saudou et al., 1998). As shown in Figure 7C, exon1 of wt htt (25Q) was seen in both cytoplasm and nucleus as well as in neurites. In contrast, exon 1 of mutant htt (103Q) was observed primarily as nuclear inclusions. In addition, overexpression of mutant but not wt htt induced cellular toxicity in cultured primary striatal neurons. However, when RAP30 was cotransfected together with mutant htt in striatal cultures, most of the nuclei of transfected cells expressing both RAP30 and mutant htt looked normal (Figure 7C). When the number of cells showing cellular toxicity was counted and calculated as a percentage of total cells expressing the transfected proteins, we found that mutant htt-mediated cellular toxicity is dramatically abrogated by overexpression of RAP30 (Figure 7D). Expression of RAP30 in the absence of mutant htt did not induce cellular toxicity. By contrast, overexpression of RAP74 alone resulted in a significant number of cells showing toxicity (Figure 7D). This toxicity level is nearly comparable to that induced by the expression of mutant htt alone. Coexpression of RAP74 with mutant htt actually resulted in more cells showing signs of cellular toxicity (Figure 7D). Thus, the presence of free RAP74 may exacerbate mechanisms that inhibit transcription of certain genes, such as the *D2* gene, leading to cell death in the HD striatum. The striking correlation between these observations in striatal neurons and our in vitro transcription studies as well as protein-protein interaction data strongly suggest that TFIIF along with Sp1 and TAF4 (TFIID) represent specific transcriptional components targeted as part of the mechanism contributing to the repression of transcription and pathogenesis in HD.

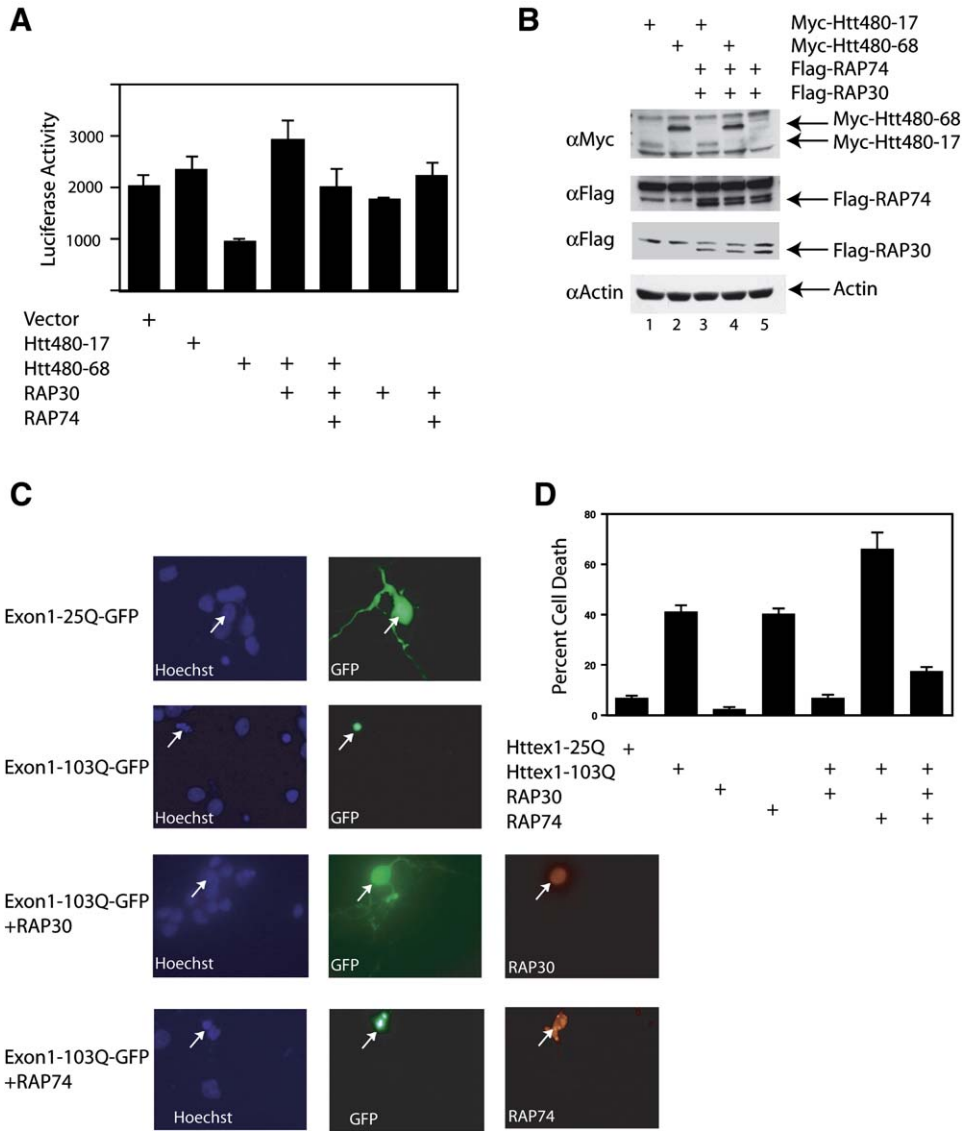

Figure 7. Mutant htt-Mediated Repression of the *D2* Receptor Promoter and Cell Death Are Prevented by Overexpression of RAP30 in Primary Striatal Neurons

(A) Effect of RAP30 on htt-induced repression of the *D2* promoter. Primary striatal neurons were transfected with D2-75Luc and expression constructs for htt (Htt480-17 and Htt480-68) and TFIIF subunit. Expression of RAP30 alone or both RAP30 and RAP74 can reverse the mutant htt-induced inhibition of *D2* promoter activity. SEM for samples were calculated from triplicates.

(B) RAP30 and RAP74 expression does not interfere with htt expression in transfected primary striatal neurons. The same amount of cell lysates was separated by SDS-PAGE and probed with αmyc antibody for myc-htt (top), αFlag antibody for Flag-RAP30/RAP74 (middle), or αActin antibody as input control (bottom).

(C) Effect of RAP30 on htt-induced cellular toxicity. Primary striatal neurons were transfected with htt (exon 1-GFP-25Q or 103Q) and/or RAP30, RAP74 expression constructs. A series of fluorescence micrographs is shown of neurons stained for DNA (Hoechst, blue, left), htt (green, middle), and RAP30 or RAP74 (red, right). Arrows indicate transfected neurons.

(D) RAP30 expression protects against mutant htt-induced cellular toxicity. Primary striatal neurons were transfected as in (B). Cell death was scored and plotted as percent cell death. SEM for samples were calculated from triplicates.

Reduced Occupancy of RAP30 at the D2 Promoter in R6/2 Mouse HD Brain

To obtain additional evidence for the involvement of RAP30/htt at a relevant target promoter, we performed chromatin immunoprecipitation (ChIP) analysis to investigate the occupancy of RAP30 at either a promoter affected by mutant htt (such as the *D2* promoter) or a control promoter that is not affected by mutant htt (such as the *NR1* promoter) in both normal and HD brain. HD brain samples were taken from 10-week-old R6/2 mice (Mangiarini et al., 1996) and normal

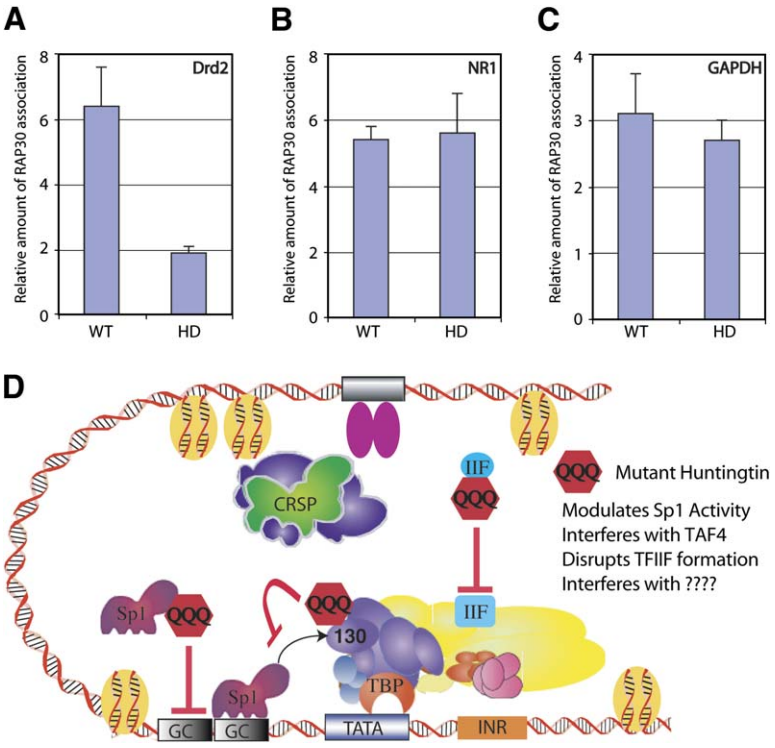

Figure 8. Reduced Occupancy of RAP30 at the *D2* Promoter in R6/2 Mice

Brain samples isolated from 10-week-old R6/2 mice (HD), and wild-type (wt) littermates were analyzed by ChIP using αRAP30 antibody or IgG as a negative control. Promoter-specific occupancy of RAP30 was analyzed by multiplex PCR. Quantitation results on the *D2* promoter (A), *NR1* promoter (B), and *GAPDH* promoter (C) were shown as graph bars. SEM for samples were calculated from triplicates. The quantitation number for IgG control ChIP was extremely low and indifferent between wt and R6/2 samples.

(D) Model of potential mechanisms used by mutant htt to disrupt Sp1-mediated transcription.

brain samples were taken form their wt littermates (wt). After isolating chromatin complexes using αRAP30 antibody, the specifically precipitated DNA was analyzed by multiplex PCR in reactions using three sets of primers specific for the core promoter regions of the mouse *D2* (*Drd2*), *NR1*, and *GAPDH* promoters. A serial 2-fold dilution of the input chromatin DNA was used to ensure the linearity of the multiplex PCR assays. As shown in Figure 8A, significantly less RAP30 is present at the *Drd2* promoter in HD brain than in wt brain. By contrast, the level of RAP30 occupancy at the control *NR1* (Figure 8B) and *GAPDH* (Figure 8C) promoter is comparable in wt and HD brain samples. This reduced occupancy of RAP30 in HD brain samples can also be detected at a number of other mutant htt responsive promoters such as the preproenkephalin gene promoter (data not shown). Our in vivo findings indicate that the level of RAP30 occupancy at promoters downregulated by mutant htt is generally reduced in HD brain, suggesting that RAP30 may represent a bona fide target of mutant htt involved in repressing transcription at early stages of HD pathogenesis.

DISCUSSION

In this study, we have developed an in vitro transcription assay to dissect the potential molecular mechanisms employed by mutant htt to repress transcription of specific promoters (e.g., Sp1-dependent). Taking advantage of this well-defined in vitro transcription system, we demonstrate that specific components (TFIID and TFIIF) of the transcriptional machinery are directly targeted by mutant htt. Importantly, these in vitro results correlate very well with the in vivo effects of mutant htt, such as the previously reported disruption of Sp1 and TAF4 interaction by mutant htt at the *D2* promoter (versus *NR1* promoter) in primary neurons (Dunah et al., 2002). Bearing this principle in mind, we may, in the future, be able to take advantage of this in vitro system to identify other potential direct targets and mechanisms of transcriptional dysregulation associated with other transcription pathways in HD. Secondly, this study demonstrates that soluble rather than aggregated forms of mutant htt may directly dysregulate transcription by interfering with specific components of the transcriptional preinitiation complex. Our data suggest that transcriptional dysfunction may occur as a result of interference by the soluble forms of mutant htt early in disease before any aggregation is seen. In addition, our work suggests that mutant htt may act as a special class of transcriptional repressor or corepressor. This is a potentially important point because it suggests that one of the primary and direct effects of mutant htt on transcription is via specific repressor mechanisms, whereas other documented effects of htt such as activation of transcription may be compensatory or secondary. Finally, our work demonstrates that transcriptional repression by mutant htt is polyQ length dependent. This strongly confirms the observed toxic gain of function for mutant htt. Progressive expansion of polyQ in mutant htt appears to lead to more severe repression while little or no repression is seen with wt htt both in vitro and in vivo. The strong correlation between polyQ length and the efficiency of repression we have observed in vitro fits well with the documented timing and severity of HD onset. This striking finding further suggests that direct disruption of

transcription integrity via aberrant interactions between mutant htt, Sp1, TFIID, and TFIIF are specific and may be significant for orchestrating the pathogenesis of HD.

In our current work, we have used a variety of different htt N-terminal fragment constructs to take advantage of the various systems established by other HD researchers. Although we realized that truncated htt proteins might behave somewhat different from the intact protein, we nevertheless believe that these in vitro and in vivo studies should be quite informative. Indeed, our in vitro studies were inspired by previous findings showing that various truncated versions of mutant htt bearing different lengths of polyQ expansions are produced by proteolytic cleavage in vivo, resulting in fragments that can readily enter the nucleus. Thus, these in vitro studies largely attempt to recapitulate the situation that is thought to occur in vivo.

The most striking finding from the in vitro studies was the identification of TFIIF as a novel direct target in mutant htt-mediated transcriptional repression. Although there have been reports linking TFIIF to the function of transcription activators and repressors (Frejtag et al., 2001), this study provides the first direct connection between TFIIF and transcriptional repression induced by a polyQ expansion protein. RAP30, a subunit of TFIIF, appears to consist of three functional domains. The N-terminal domain of RAP30 is thought to bind RAP74 (Tan et al., 1995), the central region binds RNA Pol II (McCracken and Greenblatt, 1991), and the C-terminal domain binds DNA (Garrett et al., 1992). In this study, we found that mutant htt had a strong affinity for RAP30. Because RAP30 lacks a Q-rich domain, its interaction with mutant htt is likely mediated through an alternative interface. Crystal structure of the N-terminal fragments of RAP30 and RAP74 have been shown to adopt a triple-barrel structure with multiple β sheets (Gaiser et al., 2000). Since mutant htt favors the formation of an intramolecular β sheet structure (Perutz et al., 2002), it is possible that the RAP30 mutant htt interaction involves contact between β sheet structures. Such a structure-based interference mechanism is consistent with our finding that expansion of glutamines in mutant htt enhanced its affinity for RAP30. Thus, mutant htt may target not only polyQ-containing proteins, but also non-polyQ proteins with specific β sheet structures. It should be noted that addition of Congo red, a β sheet-reactive reagent, to our in vitro system did not prevent mutant htt-mediated transcriptional repression, possibly due to its inability to prevent mutant htt from forming protofibrils in vitro (Poirer et al., 2002).

An important aspect revealed by our study is that mutant htt has a higher affinity for RAP30 than wt htt and may compete with RAP74 for interaction with RAP30. Because an intact TFIIF complex is required for efficient initiation and elongation of transcription at least for some promoters, we hypothesize that TFIIF dissociation will contribute to transcriptional dysregulation by mutant htt. It is conceivable that mutant htt, which has a higher affinity for RAP30, when it accumulates in both the cytoplasm and nucleus could cause less TFIIF to be formed in the cytoplasm and more TFIIF to be disrupted in the nucleus. Such a scenario will likely result in a general decrease of transcription in HD cells, as has been observed (Hoshino et al., 2004). In several DNA microarray studies, the level of RNA Pol II large subunit has been shown to increase in mutant HD brain (Luthi-Carter et al., 2002a). Since the role of TFIIF in transcription is dependent on its interaction with RNA Pol II, we speculate that elevated levels of RNA Pol II subunits in HD cells may arise as a compensatory mechanism triggered by decreased levels of TFIIF. However, in vitro, adding excess RNA Pol II did not rescue the htt-mediated repression.

By contrast, our findings showed that overexpression of RAP30 is able to abrogate transcriptional repression and rescue the cellular toxicity induced by mutant htt in primary striatal neurons. There are two potential explanations. One possibility is for RAP30 to interact with mutant htt and compete it away from other htt-interacting partners. Another possibility is for RAP30 to drive the formation of more TFIIF complexes, thereby potentiating transcription of important genes involved in neuronal survival. An intriguing observation we made is that overexpression of RAP74 alone could induce significant cellular toxicity in striatal neurons. This suggests that the chronic release of free RAP74 from TFIIF may contribute to the progressive nature of HD pathogenesis. Thus, our data favor the mechanism in which RAP30 can protect the striatal neurons by promoting TFIIF complex formation. To better understand how much the TFIIF-mediated mechanism contributes to the selective neuronal death during HD pathogenesis, it will be important to identify those genes whose transcription in striatal neurons is particularly sensitive to both mutant htt and RAP74 in future investigations.

Taking our in vitro and in vivo observations together with previous studies, we propose the following model for how mutant htt represses Sp1-dependent gene expression in neurons (Figure 8D). In normal cells, Sp1 is recruited to GC-box-containing promoters through its DNA binding domain. Once bound to DNA, Sp1 utilizes its multiple glutamine-rich activation domains to target components of the basal transcription machinery, one of which is TAF4, a subunit of TFIID. In a multistep recruiting process involving TFIIA, TFIID, TFIIB, TFIIE, TFIIF, TFIIH, RNA Pol II, and CRSP, the PIC is then formed on activated promoters to potentiate transcription. In HD cells, soluble nuclear mutant htt fragment is free to bind Sp1 through direct protein interactions, thus sequestering this key transcriptional activator from binding to its cognate GC boxes. Furthermore, mutant htt can also prevent Sp1-mediated recruitment of TFIID through its interaction with TAF4. In the case where there is already an Sp1-TFIID complex formed at the promoter, mutant htt could subsequently disrupt the stepwise PIC assembly by targeting TFIIF, an essential transcription factor important for initiation, promoter escape, and elongation at certain promoters. We anticipate that for different potential target genes, mutant htt will have differential effects because these multiple transcription factor targets may be differentially required for critical functions and rate-limiting transactions at specific gene promoters. In summary, this simple model describes one potential mechanism by which mutant htt can selectively target

an activator (Sp1) and multiple components of the core machinery (TFIID and TFIIF) to interfere with various stages of the transcription process. We anticipate that this model will undergo further refinements as more gene regulatory targets for mutant htt are identified and their molecular consequences determined.

EXPERIMENTAL PROCEDURES

Plasmids and Reagents

Bacterial expression constructs for His_6-tagged N-terminal human htt (1–171aa; Htt-23QP and Htt-120QP with 23 and 120 polyQ, respectively) were kindly provided by X.-J. Li (Emory University; Li et al., 2002). Bacterial expression constructs for His_6-tagged human htt exon 1 with different numbers of polyQ (25QP, 46QP, and 97QP with 25, 46, and 97 polyQ, respectively) were subcloned into pET28a (Novagen) from corresponding pcDNA3.1-Htt-exon 1-GFP constructs kindly provided by L. Thompson (University of California, Irvine). Bacterial expression constructs for various GST-htt N-terminal fragments were described (Dunah et al., 2002). cDNAs encoding the first 480 aa of human htt (Htt480-17 and Htt480-68 with 17 and 68 polyQ, respectively) and TFIIF subunits (RAP30 and RAP74) were subcloned into pCMV-Tag3B and pcDNA3-Flag vector, respectively. The *D2* luciferase construct, D2-75Luc, was generated by subcloning the *D2* promoter region from D2-75CAT into pGL3-basic vector (Promega). Antibodies against His_6-probe (sc-803), and RAP30 (sc-236) were obtained from Santa Cruz. Anti-myc antibody (9E10) was obtained from ATCC.

Expression, Purification of Recombinant Proteins, and GST Pull-Down Analysis

His_6-tagged htt fragments were expressed and purified as described (Li et al., 2002). Eluted proteins were dialyzed to HEMG buffer (25 mM HEPES [pH 7.6], 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10% Glycerol, 0.2 mM PMSF, 1 mM DTT, 1 mM sodium metabisulfite) containing 0.2 M KCl. After dialysis, precipitated proteins were removed by centrifugation, and remaining soluble proteins were frozen in liquid nitrogen and kept at −80ºC in small aliquots. GST-fusion htt fragments were expressed and purified as described (Scherzinger et al., 1997). [^{35}S]-methionine-labeled proteins were produced using TNT quick-coupled transcription/translation system (Promega). GST pull downs were performed in 100 μl HENG-0.1 M buffer (25 mM HEPES [pH 7.6], 0.1mM EDTA, 10% Glycerol, 0.1% NP40, 0.2 mM PMSF, 1 mM DTT, 1 mM sodium metabisulfite, 0.1 M NaCl) containing 0.2 mg/ml BSA and 200 μg/ml ethidium bromide for 3 hr at 4ºC. After binding, beads were washed five times, and bound proteins were analyzed by SDS-PAGE followed by immunoblotting or exposure to PhosphorImager Screen.

In Vitro Transcription

Recombinant basal transcription factors TFIIA, TFIIB, TFIIE, and TFIIF were purified and kindly provided by C. Inouye. Flag-tagged human Sp1 and Gal4-VP16 were immunopurified using M2 resin. Human TFIID, TFIIH, RNA Pol II, and CRSP were immunopurified from column fractions derived from HeLa nuclear extracts using specific monoclonal antibodies. In vitro transcription assay using chromatin templates had been described (Lemon et al., 2001; Naar et al., 1999). The purified N-terminal htt fragments were mixed with basal transcription factors on ice before the addition of completely assembled chromatin. For rescue experiments, 2- to 4-fold extra of each basal factor was added along with purified htt protein.

Neuronal Cell Culture, Transfection, and Immunocytochemistry

Striatal neurons were prepared from embryonic (E17–18) rats and cultured as described (Dunah et al., 2002). Transfection was performed using Lipofectamine 2000 (Invitrogen). For each transfection in a 12-well plate, 3.3 μl of Lipofectamine 2000 reagent and 1.8 μg of plasmid DNA were used. Transfected neurons were scored for cell death as described (Dunah et al., 2002). For reporter assay, cultured striatal cells were transfected at DIV 7–DIV 9. Luciferase activity was determined using Promega's Dual luciferase kit. For immunocytochemistry, cultured striatal neurons were transfected at DIV 4 and analyzed as described (Dunah et al., 2002). Antibodies used for staining included αGFP (Chemicon), αFlag (Sigma), αRAP30 (BD Biosciences), and αRAP74 (Santa Cruz, sc-235). All experiments were performed in triplicates and repeated at least twice. For immunoblotting, cultured striatal neurons were transfected on DIV 3. At 48 hr posttransfection, cells were rinsed with PBS and lysed in lysis buffer (50 mM TrisHCl [pH 7.4], 150 mM NaCl, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 0.5% DOC, and 1% Triton X-100). After clearing by centrifugation, 90 μg of proteins were resolved by SDS-PAGE and analyzed by immunoblotting using αFlag M2 (Sigma) or αmyc (Santa Cruz) antibodies.

Cell Culture and Coimmunoprecipitation

COS7 cells were cultured in DMEM supplemented with 10% fetal bovine serum. Transfections were performed using Lipofectamine 2000. Cells were harvested 36–48 hr later and lysed in 100 μl (for each well from a 6-well cell-culture plate) lysis buffer with complete protease inhibitor cocktail (Roche). For coIP, 80 μl of cell lysates were diluted with 320 μl binding buffer (50 mM TrisHCl [pH 7.9], 0.1% Triton X-100, 50 mM NaCl) and immunoprecipitated using αFlag or αmyc affinity resin for 4 hr at 4ºC. Beads were washed five times with wash buffer (50 mM TrisHCl [pH 7.9], 0.1 M NaCl, 0.5% DOC, 0.5% Triton X-100), boiled in sample buffer, and analyzed by SDS-PAGE and immunoblotting.

For coIP using mouse brain extracts, three striata from 4-month-old heterozygous KI mice and wt mice were pooled and homogenized with Tissuemizer in 1 ml of ice-cold CHAPS lysis buffer (40 mM HEPES [pH 7.5], 1 mM EDTA, 10 mM pyrophosphate, 10 mM β-glycerophosphate, 50 mM NaF, 1 mM PMSF, 0.3% CHAPS, and complete protease inhibitor cocktail). The tissue homogenates were briefly sonicated and cleared by centrifugation. After preclearing with protein G agarose, 1 mg of supernatant was incubated with 20 μl of protein G agarose and 4 μg of either αRAP30 antibody (BD Transduction Lab) or normal mouse IgG for 4 hr at 4ºC. Beads were then washed four times with lysis buffer and once with wash buffer (50 mM HEPES [pH 7.5], 1 mM EDTA, and 150 mM NaCl). Samples were analyzed by SDS-PAGE and immunoblotting with specific antibodies.

Chromatin Immunoprecipitation and Multiplex PCR

ChIP was performed using a ChIP kit (Upstate) according to the manufacturer's recommended protocol. Briefly, fresh brain tissues were weighted and homogenized by forcing the fresh tissue through a 22 G needle. The homogenized cell suspension was crosslinked in DMEM medium containing 1% formaldehyde for 10 min at room temperature. Chromatin was immunoprecipitated using either control serum or αRAP30 antibodies (Santa Cruz, sc-236). Immunoprecipitated chromatin was analyzed by multiplex PCR with all three sets of primer in one tube. Primers for *D2* core promoter sequences are 5′-CTGGAGCCAAAAGCAGTCTG-3′ (forward) and 5′-TCCTTCAGGTTTCCGACGCC-3′ (reverse); *NR1* core promoter sequences are 5′-CCACACGGATGACTGTCCC-3′ (forward) and 5′-GCGTTGGCGTAAATGCTTGG-3′ (reverse); *GAPDH* core promoter sequences are 5′-AAGCAGCATTCAGGTCTCTGG-3′ (forward) and 5′-TTTCCCCTCCTCCCTCTCTTT-3′ (reverse). Multiplex PCR was performed using Multiplex PCR Kit (Qiagen) for 30 cycles, with each cycle consisting of a 30 s denaturing at 95ºC, 90 s annealing at 57.4ºC, and 60 s extension at 72ºC. All PCR reactions were performed in triplicates. PCR products were analyzed and quantified by using DNA 500 Chips and Agilent Bioanalyzer (Agilent Technologies).

ACKNOWLEDGMENTS

We thank X.-J. Li and L. Thompson for various htt constructs and C. Inouye for purified recombinant transcription factors. We also thank N. Tanese and members of the Tjian Lab for their comments on the manuscript. We are grateful to J-.H. Cha and C. Benn for helpful

discussions and sharing of unpublished chromatin immunoprecipitation data. This work was funded by HHMI (R.T.) and NIH with grants to R.T. (CA025417), D.K. (R01NS050352, P01NS045242). W.Z. was supported by a postdoctoral fellowship grant from the Hereditary Disease Foundation, HighQ Foundation, and Cure Huntington's Disease Initiative.

Received: January 3, 2005
Revised: May 31, 2005
Accepted: October 18, 2005
Published: December 28, 2005

REFERENCES

Albright, S.R., and Tjian, R. (2000). TAFs revisited: more data reveal new twists and confirm old ideas. Gene *242*, 1–13.

Cha, J.H. (2000). Transcriptional dysregulation in Huntington's disease. Trends Neurosci. *23*, 387–392.

Chen, J.L., Attardi, L.D., Verrijzer, C.P., Yokomori, K., and Tjian, R. (1994). Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell *79*, 93–105.

Conaway, J.W., Shilatifard, A., Dvir, A., and Conaway, R.C. (2000). Control of elongation by RNA polymerase II. Trends Biochem. Sci. *25*, 375–380.

Courey, A.J., and Tjian, R. (1988). Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. Cell *55*, 887–898.

DiFiglia, M., Sapp, E., Chase, K., Schwarz, C., Meloni, A., Young, C., Martin, E., Vonsattel, J.P., Carraway, R., Reeves, S.A., et al. (1995). Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron *14*, 1075–1081.

DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P., and Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science *277*, 1990–1993.

Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N., and Krainc, D. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science *296*, 2238–2243.

Frejtag, W., Zhang, Y., Dai, R., Anderson, M.G., and Mivechi, N.F. (2001). Heat shock factor-4 (HSF-4a) represses basal transcription through interaction with TFIIF. J. Biol. Chem. *276*, 14685–14694.

Gaiser, F., Tan, S., and Richmond, T.J. (2000). Novel dimerization fold of RAP30/RAP74 in human TFIIF at 1.7 Å resolution. J. Mol. Biol. *302*, 1119–1127.

Garrett, K.P., Serizawa, H., Hanley, J.P., Bradsher, J.N., Tsuboi, A., Arai, N., Yokota, T., Arai, K., Conaway, R.C., and Conaway, J.W. (1992). The carboxyl terminus of RAP30 is similar in sequence to region 4 of bacterial sigma factors and is required for function. J. Biol. Chem. *267*, 23942–23949.

Gerber, H.P., Seipel, K., Georgiev, O., Hofferer, M., Hug, M., Rusconi, S., and Schaffner, W. (1994). Transcriptional activation modulated by homopolymeric glutamine and proline stretches. Science *263*, 808–811.

Hoshino, M., Tagawa, K., Okuda, T., and Okazawa, H. (2004). General transcriptional repression by polyglutamine disease proteins is not directly linked to the presence of inclusion bodies. Biochem. Biophys. Res. Commun. *313*, 110–116.

Lemon, B., Inouye, C., King, D.S., and Tjian, R. (2001). Selectivity of chromatin-remodelling cofactors for ligand-activated transcription. Nature *414*, 924–928.

Li, S.H., Cheng, A.L., Zhou, H., Lam, S., Rao, M., Li, H., and Li, X.J. (2002). Interaction of Huntington disease protein with transcriptional activator Sp1. Mol. Cell. Biol. *22*, 1277–1287.

Luthi-Carter, R., Hanson, S.A., Strand, A.D., Bergstrom, D.A., Chun, W., Peters, N.L., Woods, A.M., Chan, E.Y., Kooperberg, C., Krainc, D., et al. (2002a). Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum. Mol. Genet. *11*, 1911–1926.

Luthi-Carter, R., Strand, A.D., Hanson, S.A., Kooperberg, C., Schilling, G., La Spada, A.R., Merry, D.E., Young, A.B., Ross, C.A., Borchelt, D.R., and Olson, J.M. (2002b). Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington's disease mouse models reveal context-independent effects. Hum. Mol. Genet. *11*, 1927–1937.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W., and Bates, G.P. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell *87*, 493–506.

McCracken, S., and Greenblatt, J. (1991). Related RNA polymerase-binding regions in human RAP30/74 and Escherichia coli sigma 70. Science *253*, 900–902.

Menalled, L.B., Sison, J.D., Dragatsis, I., Zeitlin, S., and Chesselet, M.F. (2003). Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J. Comp. Neurol. *465*, 11–26.

Naar, A.M., Beaurang, P.A., Zhou, S., Abraham, S., Solomon, W., and Tjian, R. (1999). Composite co-activator ARC mediates chromatin-directed transcriptional activation. Nature *398*, 828–832.

Okazawa, H. (2003). Polyglutamine diseases: a transcription disorder? Cell. Mol. Life Sci. *60*, 1427–1439.

Perutz, M.F., Finch, J.T., Berriman, J., and Lesk, A. (2002). Amyloid fibers are water-filled nanotubes. Proc. Natl. Acad. Sci. USA *99*, 5591–5595.

Poirer, M.A., Li, H., Macosko, J., Cai, S., Amzel, M., and Ross, C.A. (2002). Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. J. Biol. Chem. *277*, 41032–41037.

Rubinsztein, D.C., Barton, D.E., Davison, B.C., and Ferguson-Smith, M.A. (1993). Analysis of the huntingtin gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of onset of Huntington's disease and CAG repeat number. Hum. Mol. Genet. *2*, 1713–1715.

Ryu, S., Zhou, S., Ladurner, A.G., and Tjian, R. (1999). The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. Nature *397*, 446–450.

Saudou, F., Finkbeiner, S., Devys, D., and Greenberg, M.E. (1998). Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell *95*, 55–66.

Scherzinger, E., Lurz, R., Turmaine, M., Mangiarini, L., Hollenback, B., Hasenbank, R., Bates, G.P., Davies, S.W., Lehrach, H., and Wanker, E.E. (1997). Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell *90*, 549–558.

Sipione, S., and Cattaneo, E. (2001). Modeling Huntington's disease in cells, flies, and mice. Mol. Neurobiol. *23*, 21–51.

Steffan, J.S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B.L., Kazantsev, A., Schmidt, E., Zhu, Y.Z., Greenwald, M., et al. (2001). Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature *413*, 739–743.

Sugars, K.L., and Rubinsztein, D.C. (2003). Transcriptional abnormalities in Huntington disease. Trends Genet. *19*, 233–238.

Tan, S., Conaway, R.C., and Conaway, J.W. (1995). Dissection of transcription factor TFIIF functional domains required for initiation and elongation. Proc. Natl. Acad. Sci. USA *92*, 6042–6046.

Tanese, N., Pugh, B.F., and Tjian, R. (1991). Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. Genes Dev. *5*, 2212–2224.

The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell *72*, 971–983.